Causal Model to Describe the Spatial and Temporal Variation of Faecal Coliform Concentrations in a Pilot-scale Test Consisting of Ponds Aligned in Series

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Moreira, J. F., Cabral, A. R., Oliveira, R. and Silva, S. A. (2009). "Causal model to describe the variation of faecal coliform concentrations in a pilot-scale test consisting of ponds aligned in series." Ecological Engineering 35(5): 791-799.

Abstract

In this study, the application of path analysis was tested to validate a causal model developed to verify the influence on several factors of the variation of faecal coliform concentration in pilot-scale ponds that treat domestic wastewater under distinct physical and operational characteristics. The ponds, aligned in series, are located in the city of Campina Grande, Northeastern Brazil. The model proved to be efficient for describing the variation in both secondary facultative and maturation ponds. The coefficient of determination (R^2) varied between 0.66 and 0.84 for the facultative ponds and between 0.54 and 0.95 for the maturation ponds. Effluent faecal coliform concentration was negatively affected by solar radiation in shallow ponds and positively affected by influent faecal coliform concentration in deep ponds. Typically the effects related to phytoplankton photosynthetic activity were well characterized in all shallow maturation ponds. Faecal coliform concentration was negatively affected by pH in almost all the cases. This model is not suitable for anaerobic ponds.

Keywords

Causal model, faecal coliform, path analysis, ponds in series, waste stabilization ponds

Résumé

La méthodologie du *Path analysis* a été employé afin de tester un modèle causal qui a été développé pour vérifier l'influence de plusieurs facteurs sur la variation de la concentration en coliformes fécaux dans des étangs de traitement des eaux usées pilotes. Ces dernières sont opérées selon des caractéristiques physiques et fonctionnelles distinctes. Les étangs, alignés en série, se trouvent dans la ville de Campina Grande, dans le Nordeste brésilien. Le modèle s'est révélé efficace pour décrire la variation de la concentration de coliformes fécaux dans les étangs secondaires facultatifs, de même que dans les étangs de maturation. Le coefficient de détermination (R^2) a varié de 0,66 à 0,84 dans étangs facultatifs et de 0,54 à 0,95 dans les étangs de maturation. La concentration en coliformes fécaux de l'effluent a été négativement affectée par les radiations solaires dans les étangs peu profonds et positivement affectée par la concentration en coliformes fécaux de l'influent dans les étangs profonds. Habituellement, les effets associés à l'activité de photosynthèse du phytoplancton étaient bien caractérisés dans les étangs de maturation peu profonds. La concentration en coliformes fécaux a été négativement affectée par le pH dans presque tous les cas. Ce modèle ne s'applique aux étangs anaérobies.

Mots-clés

Modèle causal, coliforms fécaux, série d'étangs, étangs de stabilisation

Introduction

Wastewater treatment in ponds is ordinarily described on the basis of pollutant removal efficiency. Each pond in a series is a unique ecosystem possessing particular ecological characteristics suitable for the development of a adapted biocenosis. The ecological succession observed throughout a series of ponds, is influenced by physical, operational and environmental factors. Therefore, in order to improve removal efficiency, it is, necessary to understand – for a particular system – which main factors or parameters affect the pollutant removal process.

The effects of these factors on faecal indicator bacteria have been discussed in the literature (Trousselier et al. 1986; De Oliveira 1990; Curtis et al. 1992a; Curtis et al. 1992b; Bahlaoui et al. 1998). Predominantly, the approach is to analyse isolated factors rather than to consider an interrelationship of factors. Multivariate statistical techniques are also used for data analyses in the case of pond monitoring systems (De Oliveira 1990; Gomez et al. 2000). These techniques are based on a large number of variables that help describe particular aspects of the treatment, thus contributing to establishing more rational criteria for the design of pond systems, as well as for controlling their operation.

Path analysis is one such multivariate technique; in fact, it is an extension of multiple regression. It was initially developed for data analysis in the field of genetics (Trousselier et al. 1986) but has been applied to several other areas of study, including wastewater treatment. Its application consists in the search of causal effects amongst the investigated variables in order to establish a causal model that must be based on scientific knowledge. This knowledge also helps to indicate which variables represent causes or consequences within the model. The respective influences of variables are represented by unidirectional arrows or curves in an illustrative diagram.

Murray (1988) proposed a simplified path causal model for faecal coliform removal in some ponds operating under the semiarid climatic conditions of Northeastern Brazil, where the average temperature is 25º C, with a maximum and minimum of 36º C and 15º C, respectively. The model is designed around four variables: solar radiation (SR), temperature (T), photosynthetic activity (PA) and faecal coliform decay (FCD). It also takes into account the indirect effect of solar radiation on temperature (T) and photosynthetic activity (PA). The causal hierarchy proposed by Murray (1988) is as follows: SR≥T≥PA≥FCD, where "≥" indicates the direction of the effects, rather than a mathematical symbol.

Causal models that describe the effects of a greater and more representative number of variables on effluent FCD from waste stabilization ponds have been proposed by Bahlaoui et al. (1998). The latter applied path analysis to study the simultaneous effects of pH, solar radiation, temperature, dissolved oxygen and chlorophyll "*a*" on the concentration of faecal bacteria in high-rate oxidation ponds. They demonstrated that these factors are interrelated; however their relative importance varies with the seasons and pond configuration (design). It may also vary from one year to another, depending, for example, on the total amount of solar radiation, precipitation or other significant climatic data.

The aim of the present study is to test the application of path analysis to validate a proposed causal model for the description of the variation of effluent faecal coliform concentrations in facultative and maturation ponds. The ponds, built in series, are part of a pilot scale project. The ponds are fed with domestic wastewater from the municipal sewerage system of Campina Grande, a city located in the Northeastern State of Paraiba, Brazil. The series of ponds had different physical and operational characteristics. In the proposed model, it was possible to test the influence of the different physical and operational characteristics of the ponds on effluent faecal coliform concentrations.

The Experimental Systems and Methodologies

Data were obtained from two pilot-scale experimental systems operated by EXTRABES (Federal University of Paraiba's Experimental Station for Biological Treatment of Sewage). Both systems include five ponds placed in the following serial order: one anaerobic pond fed with raw sewage, followed by a secondary facultative pond and three maturation ponds.

System I, made up of shallow ponds (1-m deep; the anaerobic pond is 1.25-m deep), has a total hydraulic retention time of 29.1 days. System I was previously described by Silva

(1982). The data analysed in the present study were obtained from an experimental program that started in February 1978 and ended in January 1979.

System II, made up of deep (2.2 m deep) ponds aligned in series, was described by De Oliveira (1990). The data of System II were analysed based on the results of two experiments: experiment 1 that lasted from January to December 1986 and experiment 2, undertaken from January to December 1987. The difference between experiments 1 and 2 was the total hydraulic retention times, which were 25 and 40 days, respectively, and the superficial organic loads, which were 677 and 328 Kg BOD_5/ha.day.

In each experiment a set of meteorological, physico-chemical and microbiological data was obtained. In the present study, the selected variables were chlorophyll "*a*" (CLA), BOD_5, influent (IFC) and effluent (EFC) faecal coliform concentrations, dissolved oxygen (DO), pH, temperature (T), solar radiation (SR), organic loading (OL) and the cumulative hydraulic retention time (HRT). The HRT is the time from the entrance of the system to the exit of the pond being analysed.

Routine monitoring of the two systems involved collection of grab samples of raw sewage and pond effluents at 8 a.m. Physico-chemical analyses were performed on a weekly basis, whereas faecal coliform determinations were made twice a week. Solar radiation was recorded once a day. Solar radiation was measured using a solar radiation integrator device (Gunn-Bellani), installed in accordance with the recommendations of The Brazilian Meteorological Institute (7).

The methods adopted to measure temperature, dissolved oxygen (electrometric), pH (electrometric), BOD_5 (dilution in standard BOD bottles) and faecal coliform (Standard filtration membranes incubated at 44.5 ºC on Difco m=FC broth) were those described in APHA et al. (1975) and APHA et al. (1980) for Systems I and II, respectively. Chlorophyll "*a*", which is used to measure photosynthetic activity, was measured by spectrophotometry in acetone extracts, following a procedure described by Konig (1984). All statistical analyses were carried out using the software package Statistics (5^{th} version, 1997) and a common spreadsheet.

The Proposed Model and Statistical Analyses

The causal model illustrated in Figure 1 was designed to test the influence of physical, environmental and operational factors on effluent faecal coliform concentrations. Its conception was based on a scientific framework that describes faecal coliform removal in facultative and maturation ponds in the literature (Trousselier et al. 1986; De Oliveira 1990; Curtis et al. 1992a; Curtis et al. 1992b; Bahlaoui et al. 1998).

Each arrow in diagram 1 represents a hypothetical causal relationship between two variables. The arrow is represented only when a statistically significant coefficient is obtained. A positive relationship means that an increase in one variable will lead to an increase in the other variable. Accordingly, a negative relationship means that a decrease in one variable leads to a decrease in the other variable. If no relationship exists, the arrow is removed from the model (see explanations of fig 2).

The model is able to test each hypothesis individually; i.e. if there is indeed a cause-effect relationship between two variables. The model also tests the direct and indirect effects of each relationship on the answer variable. For example, SR has a direct effect on EFC but also affects the latter indirectly by means of its effect on CLA and T, which ultimately affect EFC.

Numerous multiple regression analyses were performed to determine direct and indirect coefficients of causal covariance. Only statistically significant coefficients (obtained from testing each relationship) were considered. The sum of direct and indirect coefficients is equal to the path coefficient (total causal covariance). The latter is ultimately compared to the total covariance; the lower the difference between them, the more accurately the path analysis is able to explain the cause-effect relationships between the selected variables on the answer variable. This is better explained in the interpretation of the results presented in Tables 1 to 4. The model was tested for monitoring data from secondary facultative and maturation ponds in System I (experiment 1) and System II (experiments 1 and 2).

For the application of statistical analyses, sets of raw data were first reduced by estimating monthly means, which helped to homogenize the sets. In fact, it is necessary to have a dataset containing an equal number of values for each variable (Sokal and Rohlf 1981). All data samples (raw and reduced) were tested for normality using the

Kolmogorov-Simonov's goodness of fit test. Comparisons between the Pearson's correlation coefficient and that obtained from Kendall's *tau* matrices were carried out to check for linearity. It is important to emphasize that the majority of data samples complied with normality, with the exception of influent faecal coliform for System I and experiments 1 and 2 of System II, when analysed together. As a consequence, a $\log_{(x+1)}$ transformation was carried out to normalize the dataset of these experiments.

After each dataset was homogenized, they were standardized by deducting the average of each dataset from the value of each variable and the result was divided by its standard deviation. As a consequence, the effect of scale of each variable could be eliminated. The standardized dataset for every pond in each experiment as well as the combined dataset for experiments 1 and 2 in system II, were analysed. Ridge regression (Hocking 1976) was applied to avoid errors due to multicolinearity in the estimation of path coefficients. This estimation was carried out at levels of significance of less than 10% (Trousselier et al. 1986; Gomez et al. 2000). In this work, the coefficient of determination (R^2) was used to determine the fraction of the covariance of the variable effluent coliform faecal concentrations explained by the model.

Results and Discussion

Table 1 presents significant causal covariances ($\alpha < 10\%$) between pairs of variables in the shallow ponds of System I, while Tables 2, 3 and 4 present significant causal covariances obtained from experiments 1, 2 and both experiments combined for System

II, respectively. In these tables, the total covariance values, in the column labelled **A**, were obtained from the respective variables correlation matrix. Columns **B, C** and **D** show the direct, indirect and total causal covariances, respectively. The latter were estimated by ridge regression, where λ is the ridge estimator. Column **E** shows the values for non-causal variance i.e. the percentage of total covariance that the model is not able to explain, (difference between columns **A** and **D**). In column **A**, only pairs of variables with significant causal covariance in each reactor in the series of ponds are shown with the causal hierarchy indicator ">". Whenever applicable, the hydraulic retention time and the volumetric organic loading in each pond are presented.

Figure 2, constructed on the basis of the significant covariances in Tables 1-4 ($\alpha < 10\%$), illustrates the significant effects estimated using the causal model developed for the secondary facultative pond in System I (Fig 2a), and in System II, experiment 1 (Fig 2b), experiment 2 (Fig 2c) and experiments 1 and 2 combined (Fig 2d). Each of these schematic representations is, in fact, the general causal model reduced to the significant causal effects in the particular pond analysed. It is possible to interpret from Figure 2 that solar radiation (SR), BOD_5, influent faecal coliform concentration (IFC) and chlorophyll "*a*" (CLA) were the main independent variables affecting the variation of pH, effluent faecal coliform concentration (EFC), temperature (T), and organic load (OL).

Likewise for the secondary facultative pond, a schematic representation of causal effects can be drawn in order to describe the causal relation of several variables in others ponds.

These schematic representations are not presented herein but it is possible to draw them based on the results presented in Tables 1 to 4.

Shallow ponds (System I)

SR only directly and negatively affected EFC in the experiment carried out in System I, (shallow facultative, primary and secondary maturation ponds; Fig. 2a; Tab 1). Despite the fact that the influence of the action of the ultraviolet solar radiation on enteric bacteria is considered as the main bactericidal cause in tertiary residuary water treatment (Calkins et al. 1976; Moeller and Calkins 1980; Bahlaoui et al. 1998), it is also possible to verify this effect in the facultative pond (secondary treatment). This is possibly due to the shallow depth of this pond. The model was not able to identify any effect, direct or indirect, of SR on faecal coliform in the deep facultative pond of System II (Fig 2b, c, d; Tab 2 to 4). This is due to the fact that in deep ponds, the action of solar radiation is limited to a superficial layer. As a consequence SR does not considerably influence on bacterial populations in deeper ponds.

EFC was negatively affected by pH in all shallow maturation ponds. In the maturation ponds the pH varies between 7.0 and 9.0, with higher values obtained during periods of intense photosynthetic activity. An increase in pH causes ionization of the membrane constituents, which ultimately inactivates the enzymatic system affecting the bacterial cells (1). Pearson et al. (1987) has shown that when the pH reaches a value of 9.0, there is a significant decrease in faecal coliforms.

In the primary and secondary shallow maturation ponds, SR positively affected CLA (Table 1), while CLA positively affected pH as well as DO. Typically the effects in connection with phytoplankton photosynthetic activity were well characterized in all ponds in System I. The coefficient of determination varied between 0.64 and 0.67, confirming the model's effective performance in describing significant relationships occurring in shallow maturation ponds.

Deep Ponds (System II)

In the deep facultative ponds, only IFC positively affected EFC (Fig 2b, c, d; Tab 2 to 4). These ponds operated with high organic loads and received great quantities of wastewater containing faecal coliforms. Since HRT does not change within the datasets for experiments 1 and 2 taken separately, no relationship is expected to be established between this variable and any other. When the two datasets are combined, it can be seen that the change from 5 to 8 days negatively affects EFC: as the hydraulic time increases EFC decreases, which is what is expected to occur. IFC positively affected EFC in all deep maturation ponds under the conditions of experiment 1 with higher superficial organic load (System II). This positive effect is also observed upon analysis of the dataset from experiments 1 and 2 combined.

The results with maturation ponds in experiment 1, operated with lower HRT and higher superficial organic load, showed the occurrence of only one significant effect, in this case the effect of DO on EFC. On the other hand, the results obtained with the ponds in experiment 2 (where the HRT was higher and the superficial organic load was lower), showed that, in addition to the effect of DO on EFC, CLA, OL and pH also had an effect on EFC. The supersaturation concentrations of DO are normally found in stabilization ponds during sunny periods and result from photosynthetic activity. The formation of reactive species of O_2, including superoxide, hydrogen peroxide and/or the radical hydroxyl, are expected in environments supersaturated with DO and submitted to intensive sunlight. These reactive species result from the excessive capture of light by algal chlorophyll or by other cellular pigments (such as cytochromes, which work as photosynthetic catalysts) and may damage the DNA of microorganisms (Bahlaoui et al. 1998).

The effect of CLA on EFC was observed in secondary maturation ponds (experiment II and I and II combined), where one generally finds the highest concentrations of phytoplankton in ponds aligned in series (De Oliveira 1990). The bactericidal effect of CLA results from chemical substances excreted by algal biomass, which has the effect of an antibiotic (Bahlaoui et al. 1998). In experiment 2 and for the dataset of experiments 1 and 2 combined, EFC is negatively influenced by pH, while it can be positively or negatively influenced by OL, depending on the nutritional function that it develops. If the OL is high, there is no shortage of nutrients for microorganisms and OL has a positive influence on EFC. On the other hand, if the OL is low enough (particularly towards the end of the treatment), there might be a shortage of nutrients and the influence becomes negative. The effect of pH on EFC was explained earlier for the case of shallow ponds.

Conclusion

The path analysis technique was applied to develop a causal model that proved to be effective in describing the variation of faecal coliform bacteria in the effluent of shallow or deep secondary facultative ponds, as well as shallow or deep maturation ponds, placed in series. The experiments were performed under distinct physical and operational conditions, such as hydraulic retention time, surface organic loading and pond depth.

The coefficient of determination (R^2) varied between 0.66 (shallow pond in System I and deep pond for the dataset for experiments 1 and 2 combined) and 0.84 (deep pond, experiment 2). This means that the model was capable of explaining between 66% and 84% of the faecal coliform variation in the effluent of secondary facultative ponds. These results are comparable to values obtained using other models proposed in the literature. SR, HRT and mainly the IFC are the most significant effects on the effluent concentrations of facultative ponds.

For the maturations ponds, R^2 varied within the ranges 0.54-0.77, 0.64-0.84 and 0.67-0.95 for primary, secondary and tertiary deep maturation ponds, respectively. The model showed that, for maturation ponds, EFC is influenced by IFC, pH, DO, OL and CLA.

The operational characteristics of deep ponds in experiment 2, System II, with a retention time of 8 days, provide a higher dilution capacity (reservoir effect) than found in experiment 1, where the retention time was 5 days. In contrast, the results of experiments with these ponds did not show any effect of HRT on the variation of EFC. This indicates that increasing HRT from 5 to 8 days did not influence the final results and that there would be no reason to increase the retention time.

Solar radiation only directly and negatively affected faecal coliform bacteria in shallow ponds. In deep ponds the most important cause of variation was the influent concentration of faecal coliform. Finally, pH was the principal individual variable that caused faecal coliform decay in both types of ponds, the 2 systems and all experiments.

References

APHA, AWWA and WPCF 1975 and 1980. Standard methods for the examination of water and wastewater, 13th and 15th editions. New York: American Public Health Association.

Bahlaoui, M.A., Baleux, B., and Frouji, M.A. 1998. Effect of environmental factors on bacterial populations and community dynamics in high rate oxidation ponds, Water Environment Research, **70**: 1186-1196.

Calkins, J., Buckles, J.D., and Moeller, J.R. 1976. The role of solar ultraviolet radiation in “natural” water purification, Photochemistry and Photobiology, **24**: 49-57.

Curtis, T.P., Mara, D.D., and Silva, S.A. 1992a. Influence of pH, oxygen, and humic substances on ability of sunlight to damage fecal coliforms in waste stabilization pond water, Applied and Environmental Microbiology, **58**: 1335-1343.

Curtis, T.P., Mara, D.D., and Silva, S.A. 1992b. The effect of sunlight on faecal coliforms in ponds: Implications for research and design, Water Science and Technology **26** (7-8): 1729-1738.

De Oliveira, R. 1990. The performance of deep waste stabilization ponds in Northeast Brazil. PhD thesis, The University of Leeds, U.K.

Gomez, E., Paing, J., Casellas, C., and Picot, B. 2000. Characterization of phosphorus in sediments from waste stabilization ponds, Water Science and Technology, **42**: 257-264.

Hocking R. R. 1976. The analysis and selection of variables in linear regression, Biometrics, **32**: 1-49.

Konig, A. 1984. Ecophysiological studies on some algae and bacteria of waste stabilization ponds, PhD Thesis, Liverpool University, UK.

Moeller, J.R. and Calkins, J. 1980. Bactericidal agents in wastewater lagoons and lagoon design, Journal of the Water Pollution Control Federation, 52: 2442-2451.

Murray, A. 1988. A path analysis of data from Brazilian waste stabilization ponds. Civil Engineering Department, The University of Leeds, U. K.

Pearson, H.W. 1987. Algae associated with sewage treatment. Microbial technology in the developing world. Oxford: Oxford University Press.

Silva, S.A. 1982. On the treatment of domestic sewage in waste stabilization ponds in Northeast Brazil. Ph.D., University of Dundee (United Kingdom), Scotland.

Sokal, R.R. and Rohlf, F.J. 1981. Biometry: The principles and practice of statistics in biological research. W. H. Freeman and Company, New York.

Trousselier, M., Legendre, P., and Baleux, B. 1986. Modeling of the evolution of bacterial densities in an eutrophic ecosystem (sewage lagoons). Microbial Ecology, **12**, 355-379.

List of Tables

Table 1. Significant covariances ($\alpha < 10\%$) between pairs of variables in shallow ponds of System I

Table 2. Significant covariances ($\alpha < 10\%$) between pairs of variables in deep ponds of System II, experiment 1.

Table 3. Significant covariances ($\alpha < 10\%$) between pairs of variables in deep ponds of System II, experiment 2.

Table 4. Significant covariances ($\alpha < 10\%$) between pairs of variables in deep ponds of System II, experiments 1and 2 combined.

List of Figures

Figure 1 – Schematic representation of the proposed path model for describing faecal coliform variations in pilot-scale ponds in series in Northeast Brazil.

Figure 2. Significant causal effects in the secondary facultative pond in System I (**a**) and System II, experiment 1 (**b**), experiment 2 (**c**) and 1 and 2 experiments combined (**d**).

Table 1. Significant covariances ($\alpha < 10\%$) between pairs of variables in shallow ponds of System I

Causal effect hierarchy	Total covariance (A) ($\alpha < 5\%$)	Causal covariance (Ridge regression)			Non-causal covariance (E= A - D)
		Direct (B)	Indirect (C)	Total (D= B + C)	
F1 – Secondary facultative pond (5.5 d, 5.3 $gDBO_5.m^{-3}.d^{-1}$) – λ=0.10 – R^2 = 0.6578					
SR ≥ EFC	-0.675	-0.561	-	-0.561	-0.102
DBO_5 ≥ OL	0.665	0.599	-	0.599	0.066
SR ≥ T	0.322	0.322	-	0.322	0
M1 – Primary maturation pond (5.5 d, 3.8 $gDBO_5.m^{-3}.d^{-1}$) – λ=0.05 – R^2 = 0.6385					
SR ≥ EFC	-0.540	-0.412	-0.026	-0.438	-0.102
pH ≥ EFC	-0.438	-0.395	-	-0.395	-0.043
CLA ≥ pH	-0.638	0.638	-	0.638	0
CLA ≥ DO	-0.679	0.679	-	0.679	0
CLA ≥ OL	0.651	0.651	-	0.651	0
DBO_5 ≥ OL	0.306	0.306	-	0.306	0
SR ≥ CLA	0.798	0.616	0.094	0.709	0.089
M2 – Secondary maturation pond (5.5 d, 2.1 $gDBO_5.m^{-3}.d^{-1}$) – λ=0.05 – R^2 = 0.6444					
SR ≥ EFC	-0.371	-0.124	-0.182	-0.306	-0.065
pH ≥ EFC	-0.448	-0.427	-	-0.427	-0.021
CLA ≥ pH	0.658	0.658	-	0.658	0
CLA ≥ DO	0.597	0.597	-	0.597	0
SR ≥ CLA	0.756	0.680	0.009	0.689	0.067
M3 – Tertiary maturation pond (5.5 d, 1.6 $gDBO_5.m^{-3}.d^{-1}$) – λ=0.05 – R^2 = 0.6702					
pH ≥ EFC	-0.675	-0.210	-	-0.210	-0.465
CLA ≥ pH	0.147	0.147	-	0.147	0
CLA ≥ DO	0.108	0.108	-	0.108	0

Table 2. Significant covariances ($\alpha < 10\%$) between pairs of variables in deep ponds of System II, experiment 1.

Causal effect hierarchy	Total covariance (A) ($\alpha < 5\%$)	Causal covariance (Ridge regression)			Non-causal covariance (E= A - D)
		Direct (B)	Indirect (C)	Total (D= B + C)	
F9 – Secondary facultative pond (5.0 d, 15.0 $gDBO_5.m^{-3}.d^{-1}$) – λ=0.10 – R^2 = 0.7448					
IFC ≥ EFC	0.831	0.637	-	0.637	0.194
CLA ≥ OL	0.507	0.360	-	0.360	0.147
DBO_5 ≥ OL	0.758	0.631	-	0.631	0.127
SR ≥ T	0.681	0.681	-	0.681	0
M7 – Primary maturation pond (5.0 d, 8.6 $gDBO_5.m^{-3}.d^{-1}$) – λ=0.02 – R^2 = 0.7664					
IFC ≥ EFC	0.983	0.653	-	0.653	0.330
DBO_5 ≥ OL	0.719	0.719	-	0.719	0
SR ≥ T	0.684	0.684	-	0.684	0
M8 – Secondary maturation pond (5.0 d, 4.8 $gDBO_5.m^{-3}.d^{-1}$) – λ=0.10 – R^2 = 0.7704					
IFC ≥ EFC	0.713	0.703	-	0.703	0.010
SR ≥ T	0.682	0.682	-	0.682	0
M9 – Tertiary maturation pond (5.0 d, 2.8 $gDBO_5.m^{-3}.d^{-1}$) – λ=0.05 – R^2 = 0.9483					
IFC ≥ EFC	0.908	0.897	-	0.897	0.011
DO ≥ EFC	-0.343	-0.310	-	-0.310	-0.033
DBO_5 ≥ OL	0.568	0.510	-	0.568	0.058
SR ≥ T	0.674	0.674	-	0.674	0

Table 3. Significant covariances ($\alpha < 10\%$) between pairs of variables in deep ponds of System II, experiment 2.

Causal effect hierarchy	Total covariance (A) ($\alpha < 5\%$)	Causal covariance (Ridge regression)			Non-causal covariance (E= A - D) (% error)
		Direct (B)	Indirect (C)	Total (D= B + C)	
F9 – Secondary facultative pond (8.0 d, 7.4 $gDBO_5.m^{-3}.d^{-1}$) – λ=0.05 – R^2 = 0.8397					
IFC $\geq$ EFC	0.840	0.758	-	0.758	0.082
CLA $\geq$ OL	0.821	0.486	-	0.486	0.335
$DBO_5 \geq$ OL	0.666	0.420	-	0.420	0.246
SR $\geq$ CLA	0.746	0.681	-	0.713	0.033
M7 – Primary maturation pond (8.0 d, 3.8 $gDBO_5.m^{-3}.d^{-1}$) – λ=0.10 – R^2 = 0.5410					
DO $\geq$ EFC	-0.621	-0.479	-	-0.479	-0.142
CLA $\geq$ pH	-0.489	-0.489	-	-0.489	0
M8 – Secondary maturation pond (8.0 d, 2.0 $gDBO_5.m^{-3}.d^{-1}$) – λ=0.10 – R^2 = 0.8403					
CLA $\geq$ EFC	-0.290	-0.276	-	-0.276	-0.014
OL $\geq$ EFC	0.883	0.674	-	0.674	0.209
M9 – Tertiary maturation pond (8.0 d, 1.1 $gDBO_5.m^{-3}.d^{-1}$) – λ=0.05 – R^2 = 0.8420					
pH $\geq$ EFC	-0.567	-0.521	-	-0.521	-0.046
DO $\geq$ EFC	-0.678	-0.526	-	-0.526	-0.152

Table 4. Significant covariances ($\alpha < 10\%$) between pairs of variables in deep ponds of System II, experiment 1and 2.

Causal effect hierarchy	Total covariance (A) ($\alpha < 5\%$)	Causal covariance (Ridge regression)			Non-causal covariance (E= A - D)
		Direct (B)	Indirect (C)	Total (D= B + C)	
F9 – Secondary facultative pond – λ=0.05 – R^2 = 0.6591					
IFC ≥ EFC	0.614	0.552	-	0.552	0,062
HRT ≥ EFC	-0.378	-0.376	-	-0.376	-0.002
CLA ≥ pH	0.569	0.569	-	0.569	0
DBO_5 ≥ OL	0.750	0.670	-	0.670	0.08
SR ≥ T	0.583	0.583	-	0.322	0
M7 – Primary maturation pond – λ=0.05 – R^2 = 0.6688					
IFC ≥ EFC	0.601	0.446	-	0.446	0.155
CLA ≥ pH	0.353	0.353	-	0.353	0
CLA ≥ DO	0.408	0.408	-	0.408	0
DBO_5 ≥ OL	0.650	0.531	-	0.531	0.119
SR ≥ CLA	0.534	0.431	-	0.431	0.103
SR ≥ T	0.588	0.588	-	0.588	0
M8 – Secondary maturation pond – λ=0.10 – R^2 = 0.8591					
IFC ≥ EFC	0.845	0.503	-	0.503	0.342
CLA ≥ EFC	-0.599	-0.216	-	-0.216	-0.383
OL ≥ EFC	0.752	0.303	-	0.303	0.449
CLA ≥ DO	0.440	0.440	-	0.440	0
DBO_5 ≥ OL	0.605	0.555		0.555	0.050
SR ≥ T	0.596	0.596	-	0.596	0
M9 – Tertiary maturation – λ=0.05 – R^2 = 0.8990					
IFC ≥ EFC	0.939	0.911	-	0.911	0.028
pH ≥ EFC	-0.535	-0.282	-	-0.282	-0.253
OL ≥ EFC	-0.636	-0.280	-	-0.280	-0.356
SR ≥ T	0.615	0.615	-	0.615	0

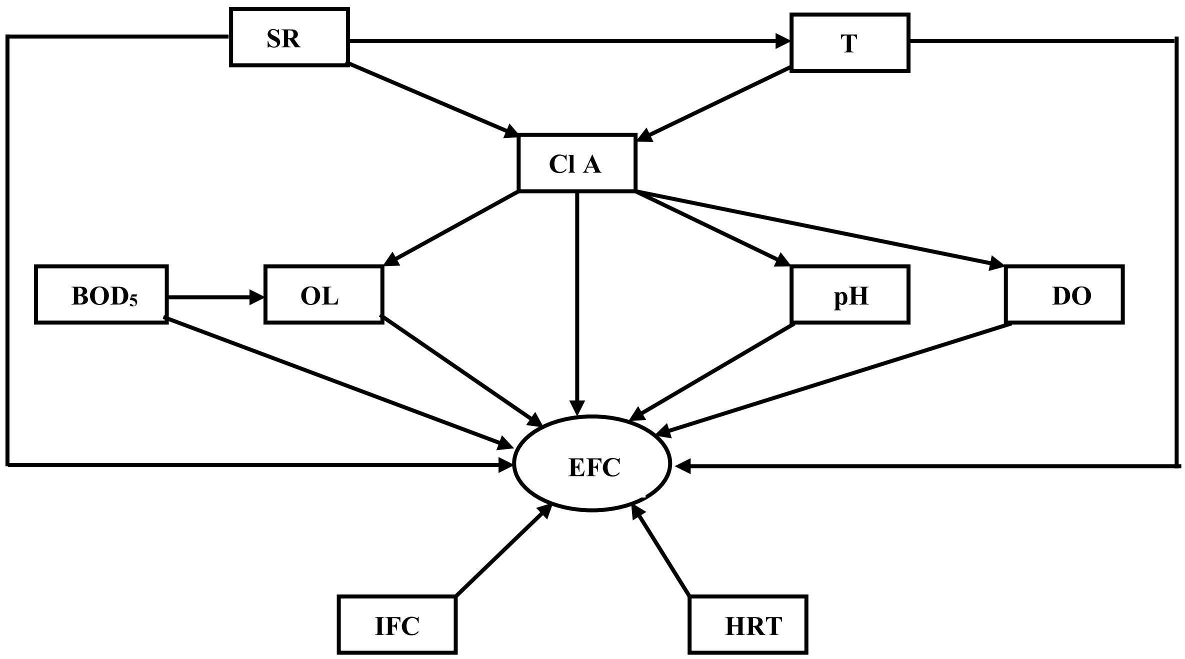

Figure 1 – Schematic representation of the proposed path model for describing faecal coliform variations in pilot-scale ponds in series in northeast Brazil.

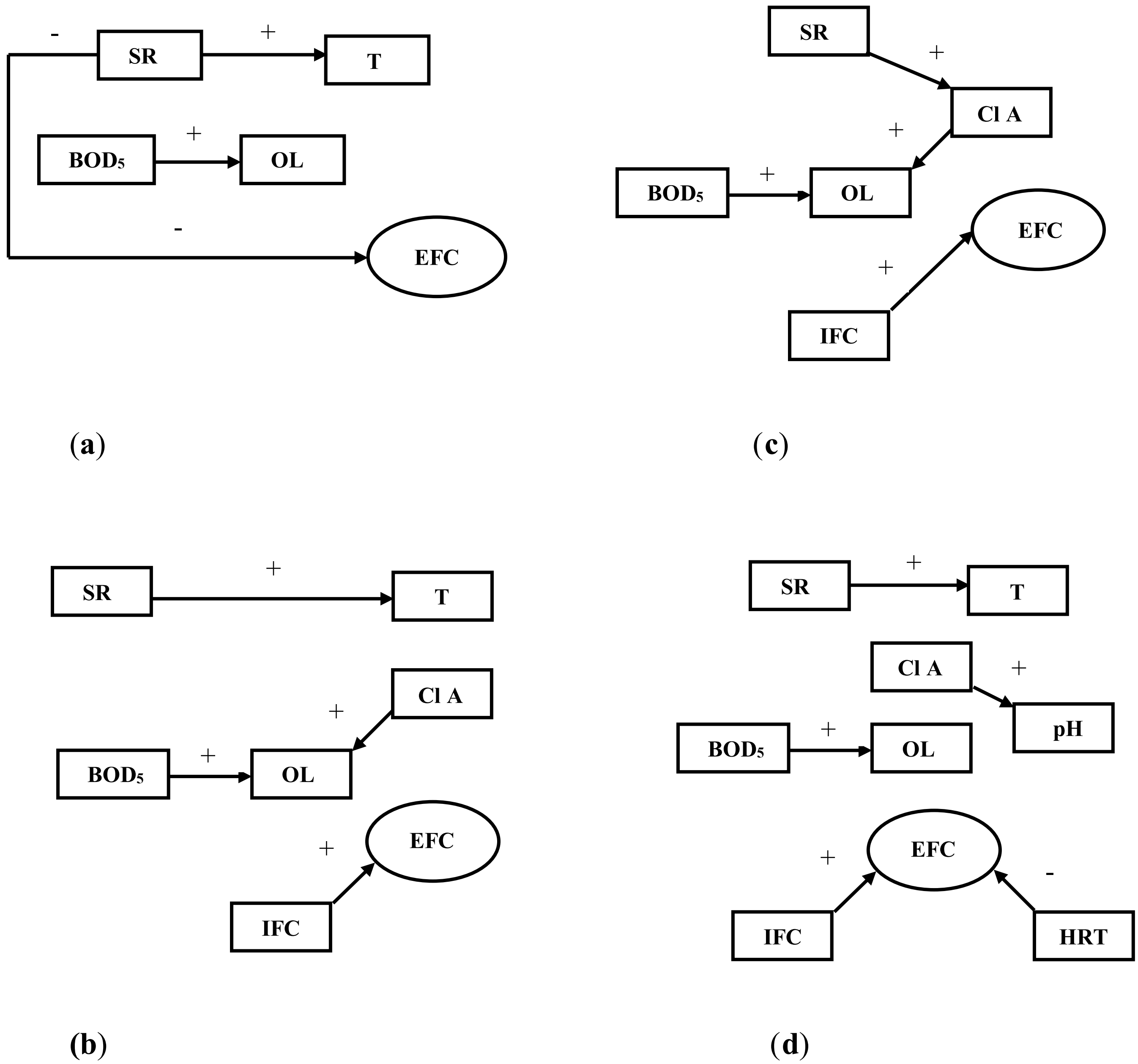

Figure 2. Significant causal effects in the secondary facultative pond in System I (**a**) and System II, experiment 1 (**b**), experiment 2 (**c**) and both experiments altogether (**c**).